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A Single-Nucleotide Polymorphism in the *Abcc6* Gene Associates with Connective Tissue Mineralization in Mice Similar to Targeted Models for Pseudoxanthoma Elasticum

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TO THE EDITOR

Pseudoxanthoma elasticum (PXE; OMIM#264800) is characterized by progressive, late-onset, ectopic mineralization of elastic fibers, clinically affecting skin, retina, and the cardiovascular system with considerable morbidity and occasional mortality (Neldner, 1988). It is an autosomal recessive disorder with a slight female preponderance and an estimated prevalence of ~1 in 50,000–70,000. The clinical diagnosis is usually made through recognition of characteristic skin lesions, i.e., small, yellowish papules on flexural areas progressively coalescing into plaques of inelastic, leathery skin. The cutaneous findings are associated with angioid streaks in the retina and mineralization of arterial blood vessels. Adding to the diagnostic difficulty is the considerable phenotypic heterogeneity in age at onset and the extent and severity of organ system involvement. Since the identification of mutations in the ATP-binding cassette, subfamily C, member 6 gene (*ABCC6*) as the genetic basis in the overwhelming majority of families with PXE, tremendous progress has been made in understanding the molecular genetics, clinical phenotypes, and pathogenesis of this disease (Uitto *et al.*, 2010).

Mutations in the *ABCC6* gene underlie the classic form of PXE, and over 300 distinct mutations representing over 1,000 mutant alleles have been reported (Uitto *et al.*, 2010). However, no apparent correlation has been established between PXE phenotypes and the nature or the position of the mutations in *ABCC6* (Pfendner *et al.*, 2007). Genetic variations in gamma-glutamyl carboxylase (*GGCX*) (Vanakker *et al.*, 2007; Li *et al.*, 2009), secreted phosphoprotein 1 (*SPP1*) (Hendig *et al.*, 2007), and xylosyltransferase I (*XYLT*) (Schon *et al.*, 2006) genes, together with environmental risk factors such as diet (LaRusso *et al.*, 2009), appear to modify the phenotype with respect to the age at onset and the extent and severity of organ involvement in PXE.

Understanding of the mechanisms underlying PXE has been advanced by the development of targeted mutant mice with genetic, histopathological, and ultrastructural features similar to those in patients with PXE (Gorgels *et al.*, 2005; Klement *et al.*, 2005). A characteristic finding in the targeted mutant mouse (B6;129S1/SvImJ-*Abcc6*^{tm1Jfk}) is the mineralization of the vibrissae dermal sheaths, the principal components of which were calcium and phosphorus (Kavukcuoglu *et al.*, 2012). This specialized hair follicle type is not found in humans, and mineralization of the vibrissae dermal sheath is very unusual in mice. The genetic background of mice can modify phenotypes associated with a single gene mutation as is the case with this *Abcc6*^{tm1Jfk}-null

Abbreviations: ATP-binding cassette, subfamily C, member 6 (gene symbol: Abcc6*, mice;* ABCC6*, human; protein symbol for both:* ABCC6*); secreted phosphoprotein 1 (gene symbol:* SPP1*, human); SUMO/sentrin-specific peptidase 6 (gene symbol:* SENP6*, human;* Senp6*, mouse); xylosyltransferase I (gene symbol:* XYLT*, human;* Xylt1 *and 2, mouse); Casr, calcium-sensing receptor; DCC, dystrophic cardiac calcinosis; PXE, pseudoxanthoma elasticum; SNP, single-nucleotide polymorphism*

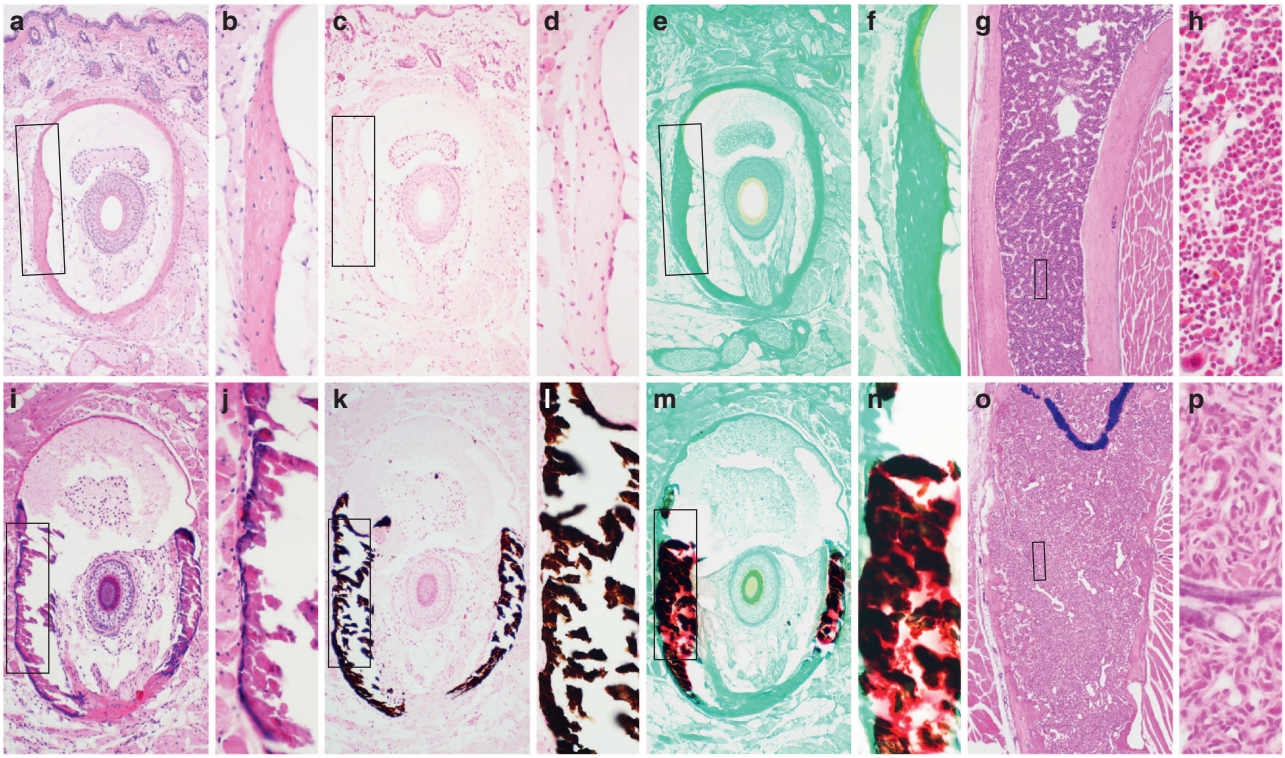

Figure 1. Vibrissae mineralization and fibro-osseous bone lesions. Sections of vibrissae from a 375-day-old female C3H/HeJ mouse have no mineralization of the connective connective tissue sheath (**a**–**f**) as demonstrated by hematoxylin and eosin staining (**a**, **b**, **i**, **g**), whereas those from a KK/HlJ mouse are mineralized (**i**–**n**), confirmed by von Kossa (**c**, **d**, **k**, **l**) and alizarin red (**e**, **f**, **m**, **n**) stains. Normal cortical bone and bone marrow from a female C3H/HeJ mouse (**g**, **h**) is very different from that of a 555-day-old female KK/HlJ mouse (**o**, **p**) with fibro-osseous lesions. Note the thin cortex and effacement of the bone marrow by fibrovascular stroma in the KK/HlJ mouse (aldehyde fuchsin stain).

mouse (Li and Uitto, 2010). Dystrophic cardiac calcinosis (DCC) in C3H/HeJ mice is also associated with a polymorphism in the *Abcc6* gene (Aherrahrou *et al.*, 2008), but these mice lack vibrissa mineralization (Figure 1). These observations suggested that spontaneous mutations in *Abcc6* may exist in inbred mouse strains and such mice would then provide novel models to study detailed mechanisms of PXE, especially for defining the role of modifier genes.

In a large-scale aging study of 31 inbred strains (Supplementary Table S1 online), mineralization of vibrissae dermal sheaths was routinely diagnosed histologically in KK/HlJ mice 6 months of age and older and in very old 129S1/SvImJ mice (Figure 1, Table 1, Supplementary Table S1 online). One case out of 68 RIIIS/J mice examined had vibrissae mineralization at the age of 20 months. Mineralization of various internal organs was observed with distinct strain distributions (Supplementary Table S2 online). Vibrissae mineralization and other PXE-like lesions (mineralization of medium-sized arteries, retina, lung, and dermis) were particularly severe in the KK/HlJ strain. KK/HlJ mice also had a number of other pathological findings, including severe fibro-osseous lesions of bones (Figure 1), as well as epicardial mineralization and fibrosis (i.e., DCC) (Table 1). The fibro-osseous bone lesion was found in a number of strains without PXE-like mineralization and was not associated with mineralization of elastic fibers, indicating that it most likely represents an unrelated disease, or that *Abcc6* was a strong modifier gene in KK/HlJ mice when mutated (Berndt and Sundberg manuscript in preparation).

Haplotype analysis of *Abcc6* revealed that one nonsynonymous single-nucleotide polymorphism (SNP) was associated with tissue mineralization. Specifically, the SNP in exon 14 (rs32756904) showed an A allele at base pair position 53,257,951 on chromosome 7 in KK/HlJ and 129S1/SvImJ—strains with vibrissae mineralization—but a G allele in strains without those lesions (Table 1). The phenotypic differences between the strain groups with the various alleles were significant (Supplementary Table S2 online). The only exception was one out of 68 RIIIS/J mice, which had vibrissae mineralization. RIIIS/J mice have a G allele at rs32756904 in exon 14. The SNP was confirmed by sequencing in all strains shown in Table 1. This SNP was recently shown in KK/HlJ mice to create a novel splice donor site, which results in the deletion of five nucleotides at the end of exon 14, causing a frameshift of the reading frame and a premature termination codon of translation (Li *et al.*, 2012), similar to previous findings in C3H/HeJ mice (Aherrahrou *et al.*, 2008).

Examination of modifier genes for human PXE, including *GGCX* (Vanakker *et al.*, 2007) (i.e., *Ggcx*), *SSP1* (i.e., *Senp6*), and *XYLT* (i.e., *Xylt1* and *Xylt2*), as well as the calcium-sensing receptor (*Casr*) in mice (Hough *et al.*, 2004), did not reveal differential SNP patterns

Table 1. *Abcc6* genotype and phenotype differences between strains

***Abcc6* genotype**

Gene	Exon	rs number	Chr	Position (bp)	Alleles ("−"strand)	129S1/SvImJ	C3H/HeJ	DBA/2J	KK/HlJ	A/J	BALB/cByJ	FVB/NJ	PWD/PhJ	C57BL/10J
Abcc6	14	rs32756904	7	53,257,951	G/A	A	A	A	A	G	G	G	G	G

Lesions														
Diagnosis						**Severity of lesions**								
Epicardial fibrosis and mineralization							+	+ + + +	+ + +	+ + +	+ + +			+ + +
Fibro-osseous bone lesions						+ + + +	+	+ +	+ + + +		+	+ +		+ +
Vibrissae mineralization						+			+ + + +					
Systemic mineralization (lung, retina)									+ +					
Arterial mineralization								+	+ + + +					

between affected and unaffected strains. DCC, characterized by mineralization and fibrosis of the heart, has been shown to be associated with four quantitative trait loci (*Dyscalc1-4*) from a cross between C3H/HeJ and C57BL/6J mice (Meng *et al.*, 2007). *Dyscalc1* is now listed as *Abcc6* (http://www.informatics.jax.org). Combined with observations that similar lesions occur in strains without the vibrissae mineralization (Table 1, Supplementary Table S2 online) and that relatively few heart lesions occur in the *Abcc6*^{tm1Jfk}-null mice (Klement *et al.*, 2005), it is likely that the cardiac lesions may not be primarily associated with *Abcc6* mutations but that *Abcc6* may rather be a major modifier of DCC phenotypes.

KK/HlJ, and to a lesser extent 129S1/SvImJ, mice provide potential new spontaneous models that may be useful for studying PXE-like phenotypes (Li *et al.*, 2012). Other lesions in these mice with various degrees of mineralization may be part of the overall pathologic process: they may reflect the expression of the influence of strain-specific modifier genes, or may represent new clinical variations.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Association of Cardiovascular and Metabolic Disease Genes with Psoriasis

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TO THE EDITOR

Psoriasis is a chronic immune-mediated and hyperproliferative disorder of the skin that affects 2–3% of the population. Psoriasis is associated with an increased incidence of several cardiovascular and metabolic comorbidities, including coronary artery disease (CAD), hypertension, obesity, hyperlipidemia, and type 2 diabetes (T2D) (Davidovici *et al.*, 2010). The association of these cardiovascular and metabolic diseases with psoriasis could be due to shared genetic risk variants, shared environmental triggers, activation of common inflammatory pathways, or a combination of these factors. Here, we evaluated the hypothesis that some of the increased risk for cardiovascular and metabolic diseases in psoriasis is derived from shared genetic risk factors.

Using the genome-wide association studies (GWAS) catalog (available at www.genome.gov/gwastudies and accessed in December 2011), we selected 363 single-nucleotide polymorphisms (SNPs) that showed significant association with CAD, hypertension, body mass index (BMI), hyperlipidemia (total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol levels and triglyceride levels), or T2D. Selected SNPs met genome-wide significance ($P<5\times10^{-8}$) in at least two GWAS or were significant in the latest meta-analysis of GWAS. Additional SNPs or loci were also included on the basis of latest expert opinions (McCarthy, 2010; O'Donnell and Nabel, 2011; Peden and Farrall, 2011). Detailed information about the selected SNPs is provided in Supplementary Table S1 online.

We evaluated the selected cardiovascular and metabolic SNPs for association with psoriasis in four psoriasis GWAS cohorts: the GAIN cohort including 1,368 psoriasis cases and 1,348 controls (Nair *et al.*, 2009), an unpublished psoriasis cohort from Sweden including 725 cases and 438 controls, a Washington University/University of California San Francisco cohort including 211 psoriasis cases and 502 controls (Liu *et al.*, 2008), and the Wellcome Trust Case–Control Consortium cohort including 2,178 psoriasis cases and 5,175 controls (Strange *et al.*, 2010). Further details of the Swedish cohort are described in the Supplementary Methods online. IMPUTE2 was used to impute the ungenotyped SNPs using phase 3 HapMap and 1,000 Genomes pilot project CEU haplotypes as a reference. SNPTEST was used to associate the imputed dosage for each SNP with psoriasis status separately in each study population with adjustment of the first three principal components from a multidimensional scaling analysis of population stratification. The association test results for these SNPs with relatively high confidence (PROPER_Info > 0.5) were then combined by meta-analysis with the META using the inverse-variance method based on a fixed-effect model. The false discovery rate method was used to correct for multiple testing (FDR_q < 0.05).

We first examined the associations between all selected SNPs and psoriasis status (Table 1 and Supplementary Table S2 online). After adjusting for multiple testing with the false discovery rate method, seven SNPs were found to be associated with psoriasis status (FDR_q < 0.05, Table 1). The alleles associated with increased risk for dyslipidemia (rs2247056, rs3177928, rs492602, and rs181362), increased blood pressure levels (rs805303, rs653178, and rs3184504), and increased risk for CAD (rs3184504) were associated with increased risk for psoriasis (Table 1). The top three SNPs (rs2247056, rs3177928, and rs805303) were located in the HLA gene region, which is a known psoriasis susceptibility locus. After further adjustment for the top psoriasis risk allele HLA-C*06:02 (determined by imputation as described in Chen *et al.*, 2012), the associations for rs2247056 and rs3177928 were mitigated ($P=0.06$ and 0.07, respectively), whereas the association for rs805303 persisted ($P=0.005$). As multiple psoriasis risk alleles are

Abbreviations: BMI, body mass index; CAD, coronary artery disease; GWAS, genome-wide association studies; HDL, high-density lipoprotein; LD, linkage disequilibrium; LDL, low-density lipoprotein; SNP, single-nucleotide polymorphism; T2D, type 2 diabetes; wGRS, weighted gene risk score